Immune response to multiple skin test antigens in haemophiliacs

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SUMMARY

Using seven skin test antigens the cell-mediated immune response was evaluated in 20 haemophiliacs, 10 human immunodeficiency virus (HIV) antibody-positive and 10 antibody-negative. Response rates were compared with 75 healthy males of similar age range. All haemophiliac patients displayed significant impairment of cell-mediated reactivity to the test antigens; however, there was no apparent correlation with HIV antibody status.

INTRODUCTION

The acquired immune deficiency syndrome (AIDS) results from destruction of a subset of cells (helper T-cells) which regulate the cell-mediated immune system. The causative agent was found to be an RNA retrovirus designated human immunodeficiency virus (HIV). Infection with this agent may result in only partial damage of the immune system and patients may, therefore, remain completely asympotamic. However, approximately 30% eventually develop the complete immunodeficiency syndrome. While virtually all subjects (>95%) who have encountered the virus develop antibodies and became HIV antibody-positive, there are as yet no methods of predicting which individuals will progress to the state of profound immunodeficiency.

Before the causative agent of AIDS was discovered, infective blood products were accidentally used to treat patients with bleeding disorders, including haemophilia, and many haemophiliacs are now HIV antibody-positive.[1]

We studied these patients to determine whether *in vivo* testing of the cell-mediated immune system could identify those who had been exposed to the HIV agent. Some workers have shown that haemophiliacs, whether HIV antibody-positive or -negative, exhibit impaired cell-mediated immune response to a specific challenge with a single antigen not previously encountered,[2] although this type of testing has a number of disadvantages.[3] Assessment of *in vivo* reactivity of the cell-mediated immune system to multiple antigens, using delayed cutaneous hypersensitivity (DCH), overcomes many of these disadvantages[4] and

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also provides information regarding the recall of immune response to antigens previously encountered by individuals as a result of infection and/or vaccination programmes. The Multitest CMI system[5] was used to study 20 haemophiliac patients, 10 of whom were HIV antibody-positive and 10 antibody-negative and 75 healthy males of similar age range.

MATERIALS AND METHODS

Study population

Twenty haemophiliac patients, age range 19 – 64 years (mean 31 years) were tested using the Multitest CMI system. Nineteen patients were suffering from Haemophilia A, while one patient had Haemophilia B (Christmas disease). All patients were asymptomatic at the time of study with none displaying any clinical evidence of immunodeficiency. The testing was performed by a single investigator (RA McI) to avoid observer error; the HIV antibody status and T lymphocyte subsets results were determined independently. Results were compared with 75 healthy males, age range 19 – 65 years (mean 36 years) who were randomly selected from the general population. Persons with concurrent or recent illness were excluded as were any subjects taking drug therapy known to cause immuno-suppression. Written informed consent was obtained from all subjects.

Multitest CMI and scoring system

The test system consists of a plastic disposable multiple puncture device capable of simultaneously applying eight test materials.[5] The antigens are applied by two rows of four puncture heads. The heads are 20mm apart so that positive reactions do not interfere with each other. Application was made to the volar surface of the forearm and skin induration was measured at 48 hours. The size of the inflammatory response was taken as the average of measurements made in two perpendicular directions. Reactions were considered positive if >2mm. The test materials include seven delayed type hypersensitivity antigens and a glycerin/saline diluent (negative control). The antigens used were two toxoids, tetanus and diphtheria, three bacterial antigens, Streptococcus, Tuberculin and Proteus, and two fungal antigens, Candida and Trichophyton.[6]

The scoring system which was used reflected the overall DCH reactivity of an individual. The score consisted of two parts: the first was the sum of the average millimetre induration for all positive responses; the second was the number of positive antigen responses out of the possible seven.

T lymphocyte subsets

At the time of the multitest CMI testing, blood was withdrawn to enumerate the proportion and absolute numbers of T lymphocyte subsets in all haemophiliac subjects. Mononuclear cells were separated by Ficoll hypaque density gradient centrifugation and the cells identified by an indirect immunofluorescent technique using monoclonal antibodies to OKT3, OKT4 and OKT8 phenotype markers for pan-T cells, helper-inducer and suppressor cytotoxic subsets respectively.

HIV antibody status

Blood was withdrawn and the presence of antibodies to the HIV virus was determined using an enzyme-linked immunosorbent assay (ORGANON).

Statistical analysis to DATA

The Mann Whitney U test was used to compare the frequency of positive reactions for each of the seven test antigens and the total millimetres of induration for each individual.

RESULTS

For the control population, the total inflammatory response ranged from 0 – 33mm with a median of 15mm. In haemophiliac patients, the range was 0 – 14mm with a median of 3mm. This was statistically significant at the 0.1% level. The number of positive responses to the antigens tested also showed a significant reduction in the haemophiliacs with a median value of 2 in this group compared with 3 for the control population; this again was significant at the 0.1% level. Overall, 99% of normal individuals reacted to one or more of the test antigens compared with 75% of the haemophiliacs, while positive reactions to two or more antigens were found in 95% and 50% respectively (Table I).

TABLE I

Number of control subjects and haemophiliacs with positive responses to test antigens

No. of positive reactions	75 Controls	20 Haemophiliacs
0	1 (1%)	5 (25%)
≥ 1	74 (99%)	15 (75%)
≥ 2	71 (95%)	10 (50%)
≥ 3	57 (76%)	4 (20%)
≥ 4	35 (47%)	1 (5%)
≥ 5	18 (24%)	1 (5%)
≥ 6	4 (5%)	0
≥ 7	1 (1%)	0

Only one of the 75 normal individuals failed to respond to any of the antigens (1.3%) while five of the 20 haemophiliacs (25%) were completely anergic. However, analysis of data comparing haemophiliacs who are HIV antibody-positive with those HIV antibody-negative showed no statistical difference for either total inflammatory response or number of positive responses. Analysis of the data for reactivity towards individual test antigens showed a marked impairment of response towards the tuberculin reagent in the haemophiliac population with only one of the 20 showing a positive response compared with 58 of the 75 normal subjects (Table II). The mean number of helper T-cells in the patients positive for antibody to HIV was 675 (range 410 – 1180) cells/mm^3 compared with a mean value of 725 (range 410 – 1010) cells/mm^3 in the HIV antibody-negative patients. This difference was not statistically significant.

All the haemophilia A patients had received cryoprecipitate and Factor VIII concentrate. The mean quantity used per patient was 51,439 units. No correlation was found between the quantity of blood product given and HIV status or degree of suppression of the cell-mediated immune response. The patient who had

received the maximum quantity of Factor VIII (347,000 units) was HIV-negative with normal CMI response, while the patient with haemophilia B had received no Factor VIII or cryoprecipitate but became HIV-positive after plasma infusion.

TABLE II

Number of control subjects and haemophiliacs with positive DCH responses to test antigens

Test antigen	75 Controls	20 Haemophiliacs
Tuberculin	58 (77%)	1 (5%)
Candida	56 (75%)	13 (65%)
Tetanus	51 (68%)	10 (50%)
Diphtheria	37 (49%)	4 (20%)
Proteus	27 (36%)	0
Streptococcus	23 (31%)	3 (15%)
Tricophyton	10 (13%)	0

DISCUSSION

The Multitest CMI system evaluates the competence of the cellular immune system *in vivo,* and in particular its ability to respond to antigens previously encountered by the individual. Our results show that all haemophiliac patients treated with blood Factor VIII concentrates have significantly impaired responses compared with a healthy age/sex matched population. No significantly detectable difference in the responses of haemophiliacs HIV antibody-positive or HIV antibody-negative could be shown and, therefore, this method of testing would not be helpful in screening haemophiliacs for possible previous exposure to the AIDS virus. There was no apparent correlation between reduced cell-mediated immune response and quantity of Factor VIII used or the presence or absence of inhibitors.

This study would confirm the findings of other workers,[1] that haemophiliac patients display impaired cell-mediated immunity. While Madhok et al[2] have recently shown this phenomenon using a single test antigen (DNCB), our data would indicate that, despite presumably similar exposure to infectious agents and vaccination programmes, haemophiliacs become anergic to previously encountered antigens or fail to develop normal recall for antigens to which they have been exposed. The lack of correlation between depressed reactivity and HIV antibody status or quantity of Factor VIII treatment would suggest that the state of impaired reactivity may not be iatrogenic, but rather an inherent defect in haemophiliacs. However, it may indicate exposure of these patients to immuno-suppressive agents other than HIV. While our study shows that haemophiliac patients fail to react positively to the tuberculin antigen, the clinical significance remains unclear. These patients were not discouraged from receiving BCG vaccination and we know of no increased incidence of tuberculosis in haemophiliacs compared with the general population.

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